Occurrence and Distributions of Hydrocarbon Oxidizing Microbes in Suspected Petroleum Bearing Site in Ahoko, Kogi State, Nigeria

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Abstract- ***The study aimed at assessing the occurrence and distribution of hydrocarbon oxidizing bacteria and fungi in a pristine and uninterrupted environment suspected to be a potent site for exploration of crude oil. Microbiological assay was used to identify the organisms present and their distributions. A total of 61 hydrocarbon oxidizing bacteria (HOB), which represented 16 different genera (3M, 9E, 3P, and 1B), was isolated across the site studied. The most widely distributed and frequently occurring organism were ethane oxidizing bacteria (EOB) which included: Achromobacter sp. (8.20%), Actinobacillus sp. (4.92%), Norcadia sp. (3.28%), propane oxidizing bacteria (POB); Mycobacterium (4.92%) and Gordonia (4.92%), butane oxidizing bacteria (BOB) identified was Ochrobacteria sp. Fifty three (53) hydrocarbon oxidizing fungi (HOF) were identified and belong to 11 genera which included: (3M, 5E, 1P and 2B), with Aspergillus sp. (20.75%) being the most widely distributed species. From the study it can be deduced that the site is widely populated by ethane oxidizing bacteria and fungi which is an indication that the site is a more potent site for gas than oil reservoir.***

Indexed Terms- ***Occurrence, distribution, hydrocarbon oxidizing microbes, exploration***

I. INTRODUCTION

The presumed oil bearing locales in Niger State, Nigeria are around the Bida Basin, situated in the North Central Region. As per Ladipo (1998), the Basins' sedimentary fill includes post-orogenic molasses and slim unfurled marine residue. The bowl is a delicately down-distorted trough whose starting point is firmly associated with Santonian orogenic developments in South East Nigeria and the Benue valley. The bowl patterns opposite to the fundamental hub of the Benue Trough and the Niger Delta Basin and is viewed as the North West expansion of the Anambra Basin, the two of which were major depocentres during the third major transgressive cycle in the Late Cretaceous (Obaje *et al*., 2011).

Oil has for the most part been recouped by oil boring which is done after investigations of auxiliary topography (at the repository scale), sedimentary bowl examination, and store portrayal have been finished (Guerriero *et al*., 2012) and one of the strategies utilized for hydrocarbon investigation, portrayal and distinguishing proof of microorganisms from hydrocarbon presumed site is regularly known as microbial prospecting (Etiope, 2015) and it depends on the way that, vaporous hydrocarbons through emission and dissemination relocate upward from subsurface oil gatherings, and are used by an assortment of microorganisms present in the sub-soil biological system. The methane, ethane, propane, and butane-oxidizing microbes solely utilize these gases as carbon hotspot for their metabolic exercises and development. These microorganisms are for the most part found enhanced in the shallow soils and can separate between hydrocarbon planned and non-imminent regions (Rasheed *et al*., 2014). In terms of

investigation aiming at the microbial hydrocarbon prospection, two groups are relevant: gram-positive bacteria, represented mainly by Actinobacteria from CRNM complex (*Corynebacterium, Rhodococcus, Nocardia* and *Mycobacterium*) that uses short-chain hydrocarbons (C_2-C_8) as an energy source, and gram-negative bacteria, mainly the genus *Pseudomonas* that possesses the ALK system responsible for alkane degradation (Shennan, 2006; Kotani *et al.*, 2006).

According to Hitzman *et al.* (2009), prospections associated with micro-seepage anomalies are 4-6 times more likely to result in a commercial discovery than prospects with no associated seepage anomaly.

The Microbial Oil Survey Technique (MOST), developed by Philips petroleum company in the 1950s (Hitzman, 1959) depends on the relationship between surface microbial anomalies. This kind of approach has proven successful in the discovery of oil and gas reservoirs both on shore and offshore (Turkiewics, 2011).

A second possibility relates to the transport of microbes from oil reservoirs via the upward flow of petroleum fluids to the seabed and into the seawater. Indigenous reservoir microorganisms may become so called hitchhiker'' cells that are carried from one environment to another, adhering to the surface of rising gas bubbles and/ or oil globules (Leifer and Judd, 2002). In the marine environment these hitchhiker may represent conspicuous indicators of this transport pathway. The hitchhiker cell phenomenon and strategy remain hypothetical and have not been reported (Leifer and Judd 2002). It is critical that the microbes targeted by either proposed strategy are appropriate for seep prospecting. Careful selection of indicator organisms will minimize the possibility of false positive while hunting for petroleum seepage (Tucker and Hitzman, 1996). The aim of the study was to assess the occurrence and distribution of hydrocarbon oxidizing microbes in suspected hydrocarbon bearing site at Ahoko, Kogi State, Nigeria.

II. MATERIALS AND METHODS

2.1 Study site

The studt site was Ahoko comprising of Ebira, Gbagi and Idu, found along Abuja-Lokoja Express Way is a local government in lokoja, Kogi State, North central Nigeria. The area is characterized by two climatic seasons: dry season (November – March) and rainy season (April – October). The inhabitants of the area are predominantly Ebira, Gwagi and few Fulani whose major occupations are farming and fishing. It lies on longitude 8° 00″N and latitude 6° 00″E (Figure.1).

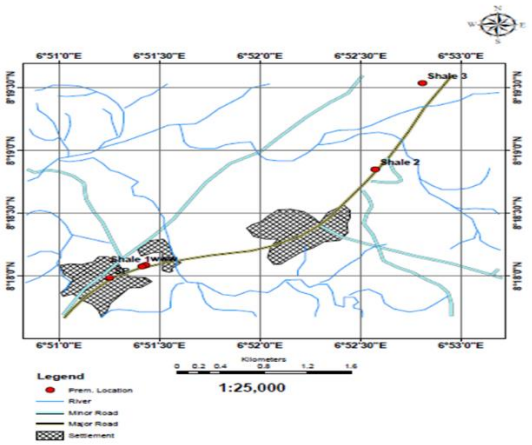

Figure 1: Map of Ahoko study site showing points of sample collection
(Victor-Ekwebelem *et al*., 2020)

2.2 Experimental Design and Sample Collection

The site was mapped and gridded within a 4km square meter. Soil samples were collected using soil core sampler by physical hammering into a depth of 1m. Each kilometre was partitioned into 5 points and samples were collected in each point and were clustered into one sample. The water samples from Mini River were collected using plastic cans which was properly washed and sterilized with 70% alcohol, then allowed to air dry. The pre-sterilized dried container was used to collect water halfway between the surface and bottom of the water (Mini river). The plastic cans were filled to the rim, leaving airspace of 2.0cm and the lid was covered immediately after collections. The soil and water samples were labelled along with their coordinates (Latitude and Longitude)

ascertained using a Global Positioning System (GPS) (Table 1), thereafter they were packed into a bucket containing ice and was transported to Microbiology Laboratory (Alex Ekwueme Federal University Ndufu Alike Ikwo (AE-FUNAI) and Step B Laboratory of the Federal University of Technology Minna, Niger State, Nigeria where samples were analysed.

Table 1: sample location and their coordinates

SITES/LOCATION	LATITUDE	LONGITUDE
A	6°40'15.3"E	8°12'30.05'N
B	6°48'10.9"E	8°15'47.00'N
C	6°51'15.3"E	8°17'51.1'N
D	6°52'47.8"E	8°19'30.5'N
WATER	6°52'47.8"E	8°19'30.6"N

2.3. Isolation and identification of isolates

The specific hydrocarbon gas oxidizing microbes were isolated using hydrocarbon gas inoculated in a vacuum desiccator at 35°C for ten days (Victor-Ekwebelem *et al*., 2020)

Isolates were identified using standard methods which include: microscopic and biochemical Analysis. The microscopic examination for bacterial isolates were done using the oil immersion objective (x100) while the fungal isolates were observed at x10 and x40 objective using hanging drop smear.

The basic biochemical test carried out were: Indole, catalase, coagulase, spore, motility, citrate, Starch utilization, oxidase, MR/VP, Glucose fermentation and H_2S production.

The specific hydrocarbon gas oxidizing microbes which included the methane, ethane, propane, butane utilizing bacteria and fungi isolates obtained were characterized and identified based on their cultural, morphological and biochemical characteristics using the scheme of Bergey's Manual of Determinative Bacteriology, online atlas and Fungi atlas respectively (Holt *et al*., 1994; Chikere and Okpokwasili, 2003; Oboh *et al*., 2006)

III. RESULTS

3.1 Identification and Frequency of Occurrence of Bacteria and Fungi in the Soil and Water samples

The most frequently occurring bacterial species in site A and B was *Micrococcus* sp. with a percentage occurrence of 5.03% and 2.79%, closely followed by *Enterobacter* sp. (4.50%, 2.23%) and *Corynebacterium* sp. (3.35%, 1.68%), while in site C the most frequently occurring organism was *Serratia* sp.(2.23%), site D and water had *Salmonella sp.at* 3.35% and 5.59% respectively (Table 2). Out of 179 bacterial isolates 23(12.83%) were *Salmonella*, 19(10.62%) *Micrococcus* and 18(10.08%) *Enterobacter*, while the least frequently occuring isolate *was Stenotrophomonas* 2(1.12%). The sites of study were dominated by *Aspergillus* species as revealed in Table 3 where *A. niger* 9(15.49), *A. flavus* 8(13.78) and *A. fumigatus* 7(12.06). Out of 58 species identified, site A and D had 22(37.93) and 9(15.53) respectively. Site B and C had 7(12. 07) each while water samples had 9(15.52%) (Table 3).

Table 2: Frequency of occurrence (%) of bacterial isolates in Ahoko soil and water samples analyzed

	Soil samples					
	SITE A	SITE B	SITE C	SITE D	WATER	TOTAL
BACTERIA						
Pseudomonas alcaligenes	3(1.68)	0(0.0)	2(1.12)	3(1.68)	3(1.68)	11(6.16)
Acinetobacter sp.	2(1.12)	0(0.0)	3(1.68)	5(2.79)	0(0.0)	10(5.59)
Enterococcus sp	4(2.23)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(2.23)

Alcaligenes sp.	3(1.68)	2(1.12)	0(0.0)	0(0.0)	0(0.0)	5(2.8)
B. licheniformis	3(1.68)	0(0.0)	0(0.0)	0(0.0)	4(2.23)	7(3.91)
Serratia spp.	2(1.12)	2(1.12)	4(2.23)	4(2.23)	0(0.0)	12(6.7)
Micrococcus sp.	9(5.03)	5(2.79)	3(1.68)	2(1.12)	0(0.0)	19(10.62)
B .coagulase	3(1.68)	5(2.79)	3(1.68)	0(0.0)	2(1.12)	13(7.27)
S. aureus	1(0.56)	2(1.12)	3(1.68)	3(1.68)	0(0.0)	9(5.04)
Corynebacterium sp	6(3.35)	3(1.68)	0(0.0)	2(1.12)	4(2.23)	15(8.38)
Enterobacter sp.	8(4.50)	4(2.23)	0(0.0)	2(1.12)	4(2.23)	18(10.08)
Salmonella sp	0(0.0)	0(0.0)	7(3.91)	6(3.35)	10(5.59)	23(12.85)
Pseudomonas sp.	3(1.68)	2(1.12)	2(1.12)	3(1.68)	3(1.68)	13(7.28)
*Citrobacter freundi*i	2(1.12)	0(0.0)	0(0.0)	0(0.0)	2(1.12)	4(2.24)
E. coli	0(0.0)	0(0.0)	0(0.0)	2(1.12)	6(3.35)	8(4.47)
Stenotrophomonas	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.12)	2(1.12)
Klebsiella sp.	0(0.0)	0(0.0)	2(1.12)	4(2.23)	0(0.0)	6(3.35)
TOTAL	49(27.40)	25(13.97)	29(16.22)	36(20.12)	40(22.34)	179(100)

Table 3: Frequency of occurrence (%) of Fungal isolates in Ahoko soil and water samples analyzed

	Soil samples (%)					
	SITE A	SITE B	SITE C	SITE D	WATER	TOTAL
FUNGI						
Aspergillus fumigatus	0(0.00)	0(0.00)	0(0.00)	5(8.62)	2(3.44)	7(12.06)
Aspergillus niger	3(5.17)	2(3.44)	2(3.44)	1(1.72)	1(1.72)	9(15.49)
Aspergillus flavus	4(6.90)	0(0.00)	2(3.44))	00(0.00)	2(3.44))	8(13.78)
Candida sp	3(5.17)	1(1.72)	1(1.72)	2(3.44)	1(1.72)	8(13.77)
Rhodotorula sp	4(6.90)	1(1.72)	0(0.00)	0(0.00)	0(0.00)	5(8.62)
Penicillium sp.	1(1.72)	2(3.44)	1(1.72)	0(0.00)	0(0.00)	4(6.91)
Rhizopus sp.	2(3.44)	0(0.00)	0(0.00)	3(5.17)	0(0.00)	5(8.61)
Alternaria sp	1(1.72)	0(0.00)	0(0.00)	0(0.00)	1(1.72)	2(3.44)
Aspergillus sp	2(3.44)	2(3.44)	0(0.00)	0(0.00)	1(1.72)	5(8.60)
Geotricum sp	2(3.44)	0(0.00)	1(1.72)	2(3.44)	1(1.72)	6(10.32)
TOTAL	22(37.93)	7(12.07)	7(12.07)	13(22.41)	9(15.52)	58(100)

3.2 Identification and Frequency of Occurrence of Specific Hydrocarbon Oxidizing Microorganisms in the SPBS

A total of 61 hydrocarbon oxidizing bacteria (HOB), which represented 16 different genera (3M, 9E, 3P, 1B) was isolated across the site studied. The most frequently occurring bacteria was ethane oxidizing

bacteria (EOB) which included *Achromobacter* sp. (8.20%), *Actinobacillus* sp. (4.92%), *Norcadia* sp. (3.28%), propane oxidizing bacteria (POB)*; Mycobacterium* (4.92%) and *Gordonia* (4.92%), and butane oxidizing bacteria (BOB) identified was *Ochrobacteria sp.(*4.92%) (Table4). Fifty three (53) hydrocarbon oxidizing fungi (HOF) were identified and belong to 11 different genera which included: (3M, 5E, 1P and 2B), with *Aspergillus* sp. (20.75%) being the most frequently occurring species (Table 5).

Table 4: Frequency of occurrence (%) of specific hydrocarbon gas oxidizing bacteria in soil and water samples analyzed

BACTERIA	Soil samples					HC-Gas	Total
	SITE A	SITE B	SITE C	SITE D	WATER		
Mycobacterium sp	3(4.92)	0(0.00)	0(0.00)	0(0.00)	2(3.28)	P	5(8.20)
Actinobacillus sp	3(4.92)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	E	3(4.92)
Norcadia sp	0(0.00)	2(3.28)	1(1.64)	0(0.00)	2(3.28)	E	5(8.20)
Enterobacter sp	1(1.64)	1(1.64)	2(3.28)	0(0.00)	0(0.00)	E	4(6.56)
Sporosarcina sp	0(0.00)	0(0.00)	0(0.00)	1(1.64)	0(0.00)	M	1(1.64)
Gordonia sp	3(4.92)	0(0.00)	1(1.64)	0(0.00)	2(3.28)	P	6(9.84)
Achromobacter sp	5(8.20)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	E	5(8.20)
Serratia sp.	1(1.64)	1(1.64)	1(1.64)	2(3.28)	0(0.00)	E	5(8.20)
Methylomonas sp	2(3.28)	1(1.64)	0(0.00)	0(0.00)	2(3.28)	M	5(8.20)
UNIDENTIFIED	3(4.92)	1(1.64)	0(0.00)	1(1.64)	4(6.56)	P	9(14.76)
Stenotrophomonas	0(0.00)	0(0.00)	2(3.28)	0(0.00)	0(0.00)	E	2(3.28)

Geobacter sp	0(0.00)	0(0.00)	0(0.00)	0(0.00)	4(6.56)	E	4(4.65)
Aeromonas sp	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(1.64)	E	1(1.64)
Methylobaceter sp	0(0.00)	0(0.00)	0(0.00)	0(0.00)	3(4.92)	M	3(4.92)
Ochrobacteria sp	0(0.00)	0(0.00)	0(0.00)	0(0.00)	3(4.92)	B	3(4.92)
Arthrobacter sp	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(1.64)	E	1(1.64)
TOTAL	21(34.4)	6(9.84)	7(11.48)	4(6.56)	24(39.34)		61(100)

Key: M=methane P=propane, E=ethane, B=butane gas

Table 5: Frequency of occurrence (%) of specific hydrocarbon gas oxidizing Fungi in soil and water samples analyzed

	Soil samples						
FUNGI	SITE A	SITE B	SITE C	SITE D	WATER	HC-Gas	Total
Aspergillus sp.	11(20.75)	4(7.55)	2(3.45)	2(3.45)	2(3.45)	E	21(39.62)
Aspergillus niger	4(7.55)	1(1.89)	1(1.89)	1(1.89)	1(1.89)	E	8(15.09)
Geotrichum sp	2(3.45)	0(0.00)	0(0.00)	0(0.00)	2(3.45)	P	4(7.55)
Penicillium sp	4(7.55)	1(1.89)	2(3.45)	1(25.00)	1(1.89)	M	9(16.98)
Rhizopus sp	1(1.89)	1(1.89)	0(0.00)	0(0.00)	0(0.00)	E	2(3.45)
Pichia sp	1(1.89)	0(0.00)	1(14.28)	0(0.00)	0(0.00)	B	2(3.45)
Rhodotorula sp	1(1.89)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	M	1(1.89)
Talaromyces sp	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(1.89)	B	1(1.89)
Alterneria sp	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(1.89)	E	1(1.89)

Aspergillus flavus	1(1.89)	0(0.00)	0(0.00)	0(0.00)	1(1.89)	M	2(3.45)
Candida sp	3(10.71)	0(0.00)	1(14.28)	0(0.00)	0(0.00)	E	4(7.55)
TOTAL	27(50.94)	7(13.21)	7(13.21)	4(7.55)	9(16.98)		53(100)

Key: M=methane P=propane, E=ethane, B=butane gas

3.3 Distribution of Microbes in Study Site

The results of the percentage distribution and relative abundance of the hydrocarbon gas utilizing microbes in soil and water samples analysed revealed that MOB: 4.92% (Site A), 1.64 (Site B and D); 9.84% (Site A), 4.92% (Site B), 8.20(Site C) and 1.64 (site D) and 16.39% (water) (Figure 2). Also the MOF were:3.85%, 1.92%, 0.00% and 0% and 1.92%; EOF, 40.38%, 1.92%, 3.85%, 5.77 and 7.69% for site A, B, C, D and water samples respectively (Figure 2).

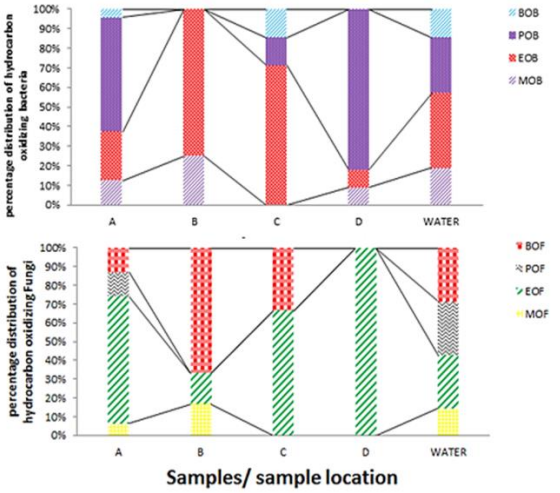

Figure 2: Relative distribution of HOB and HOF (hydrocarbon oxidizing microbes) in Ahoko soil and water.

IV. DISCUSSION

The study assessed the presence of bacteria and fungi in the soil and water sample from Ahoko area suspected to be a potent petroleum bearing site, also the hydrocarbon oxidisers in bacterial and fungal species was also evaluated which is a specific pointer to potent sites.

The dominant and most frequently occurred organisms were *Micrococcus* sp. (18.7% and 20.0%), *Enterobacter* sp. (16.33%, 16%) *Corynebacterium* sp. (12.24%, 12%), and *Serratia* sp. (13.8%) (CENM)(Table 2). According to some researchers (Shennan, 2006; Kotani *et al.*, 2006), who conducted investigations aimed at the microbial hydrocarbon prospection, two groups of microbes are relevant: gram-positive bacteria, represented mainly by Actinobacteria from CRNM complex (*Corynebacterium, Rhodococcus, Nocardia* and *Mycobacterium*) that uses short-chain hydrocarbons (C_2-C_8) as energy source, and gram-negative bacteria, mainly the genus *Pseudomonas* (Shennan, 2006; Kotani *et al.*, 2006). Following this statement, it means that, site A (Ahoko area) of this study are most likely to be the hydrocarbon potent sites, because all the aforementioned CRNM complex except R (*Rhodococcus*) were frequently present in the samples (Table 2).

The specific hydrocarbon gas oxidizing microbes (HOM) were identified and separated as hydrocarbon oxidizing bacteria (HOB) and hydrocarbon oxidizing fungi (HOF). The results revealed 16 HOB (Table 4) and 11 HOF (Table 5). Out of the 16, 9 were EOB which belong to the genera: *Enterobacter, Achromobacter, Serratia, Stenotrophomonas, Geobacter, Aeromonas, and Arthrobacter, Actinobacillus, Norcadia,* 3 MOB: *Sporosarcina, Methylomonas, Methylobaceter*, 3 POB, *Mycobacterium, Gordonia,* **unidentified** and 1 BOB *Ochrobacteria* (Table 4). For the 11 HOF, 5 were EOF which included, *Aspergillus* spp., *Aspergillus niger* , *Rhizopus, Alterneria, and Candida*, 3 MOF *Penicillium, Rhodotorula, Aspergillus flavus*, 1 POF;

Geotrichum and 2 BOF; *Pichia* and *Talaromyces* (Table 5). Shennan (2006) and Allamin *et al.* (2014) isolated similar fungi isolates from Kukawa (Nigeria) oil exploration site. Molly *et al.* (2014) stated that less was known about the organisms that oxidized ethane or propane in the environments, but a number of such isolates were primarily represented by high G+C Gram-positive bacteria which include *Nocardia*, *Pseudonocardia*, *Gordonia*, *Mycobacterium*, and *Rhodococcus* with limited *Pseudomonas* species (Shennan, 2006). These organisms have similarly been isolated in the present study.

The presence of MOM is a sparing pointer to a potent site, but the high relative abundance and wide distribution of EOM (which are oxidizers of ethane gas C_2) and POM (oxidizers of propane gas C_3) especially in site A samples confirmed the potency of these sites for oil exploration (Shennan, 2006; Kotani *et al.,* 2006; Victor-Ekwebelem *et al*., 2020), however, the production may not be in large commercial quantity because the total counts were less than 10000ppm as affirmed by Rasheed *et al*. (2015).

The cross-plots between C_1, C_2, C_3 and C_4 samples from Ahoko area revealed by the line series in the relative distribution and abundance result, displayed accurate correlation (r = 0.9) with a relationship ratio between MOM and MOF of 50% (Figure 2), the results imply that the hydrocarbons might have been generated from a thermogenic source. These interpretations follow the genetic diagram for correlating fuel wetness, reported by some researchers (Fred *et al*., 2017; Perez-Drago *et al*., 2019; Sowizdzal *et al*., 2020) who used $C_1/ (C_2 + C_3)$ ratios with the 13C of methane to categorise natural gasoline types as biogenic or thermogenic. The investigators reported that ratios of $C_1/ (C_2 + C_3)$ less than 50% are usual for thermogenic hydrocarbon gases with values among 25% and 50% whereas the ratios of $C_1/ (C_2 + C_3)$ above a thousand with values between 60% and 85% are indicative of biogenic origin of hydrocarbon gases.

The distribution map constructed to delinate the hydrocarbon potent zone revealed that site A harboured more of the hydrocarbon oxidizing microbes from Ahoko, and widely distributed with EOM and POM. According to Rasheed *et al*. 2018, there is a direct positive relationship between the increased hydrocarbon concentrations and increased hydrocarbon indicating microbial populations. With reference to the above statement it implies that, Site A of Ahoko zones of this study is the most probable site for hydrocarbon exploration.

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